

Early Preventive Effects and Mechanism of Xianling Gubao on Stress Fractures

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Abstract

Objective: Stress fractures are highly prevalent among military personnel and endurance athletes under excessive mechanical loading. Existing therapeutic strategies predominantly focus on symptomatic intervention and rehabilitation after the onset of stress fractures, whereas effective early preventive approaches remain insufficient. The traditional Chinese medicine (TCM) theory of “the kidney governs bone” provides a novel theoretical basis for the proactive prevention of stress fractures. Xianling Gubao (XLGB), a classic kidney-tonifying proprietary Chinese medicine, is widely applied in clinical practice for the treatment of osteoporosis and the promotion of fracture healing. Nevertheless, the efficacy and underlying mechanism of XLGB in stress fractures prevention have not been fully elucidated. **Method:** Five-month-old male SD rats were pretreated with different XLGB regimens, followed by two weeks of axial fatigue loading to establish ulnae stress fracture models to investigate its early protective effects and preliminary mechanisms. SPECT/CT scanning was used to detect radionuclide uptake, and alkaline rose staining was performed to assess linear microcracks, diffuse bone microdamage, and canalicular connectivity for identifying the optimal intervention protocol. H&E staining, toluidine blue staining, TRAP staining and three-point bending tests were applied to evaluate the activities and functions of osteocytes, osteoblasts and osteoclasts, as well as biomechanical properties of bone tissues. Additionally, Caspase-3/RANKL immunofluorescence double staining was used to detect the apoptosis of bystander osteocyte and osteoclasts recruitment. Runx2 immunohistochemistry was conducted to quantify osteoblasts recruitment and evaluate bone remodeling capacity. **Result:** i) Early XLGB intervention significantly decreased ^{99m}Tc-MDP uptake and alleviated bone microdamage (including linear microcracks and diffuse microdamage) in the rat ulnae, while notably improving canalicular connectivity. ii) Under cyclic fatigue loading, XLGB treatment increased trabecular bone mass, suppressed

excessive osteoclast activation, and elevated osteoblastic activities, thereby maintaining the bidirectional homeostasis of bone metabolism. Additionally, XLGB significantly reversed the fatigue-induced reduction of ultimate load and stiffness in rats' ulnae. iii) Mechanistically, early XLGB intervention attenuated apoptosis of bystander osteocytes, facilitated the recruitment of osteoclasts and osteoblasts, and augmented targeted bone remodeling. Such bidirectionally modulation of bone remodeling homeostasis effectively enhanced the microdamage repair capacity of ulnar bone tissue. Conclusion: Early prophylactic intervention with XLGB effectively ameliorates fatigue-induced bone microdamage and biomechanical deterioration by balancing bone metabolism, mitigating bystander osteocyte apoptosis and coordinating osteoclasts and osteoblasts recruitment to regulate targeted bone remodeling homeostasis. Accordingly, XLGB exerts prominent preventive effects against stress fractures. The present findings provide a viable TCM strategy for the early protection of stress fractures.

Keywords

Stress Fracture, Xianling Gubao, Bone Microdamage, Bone Remodeling, Osteocyte, Bone Metabolism